SYNTHETIC GENOMICS®

Standard Operating Procedure	Date: 05-Apr-2019	Version: 1
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SOP Name: Microalgae Inactivation Study		
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1. **Purpose**
   1.1. The purpose of this SOP is to detail how to perform an inactivation (aka "kill") study for microalgae.

2. **Hazard Identification and Risk of Exposure to the Hazards**
   2.1. *Do not ingest untested cultures or unreleased materials.*
   2.2. *Chlorine Bleach (4.0% sodium hypochlorite)* **– NFPA Rating:** (estimated) Health: 3; Flammability: 0; Instability: 0; **Danger!** Corrosive. Causes eye and skin burns. Causes digestive tract burns. Harmful if inhaled. Causes respiratory tract irritation. May cause methemoglobinemia. **Target Organs:** Eyes, Skin, Lungs, Blood.

3. **Safety Equipment and Personal Protection Equipment (PPE)**
   3.1. Safety glasses, nitrile gloves, and full-length clothing.

4. **Waste Generation and Disposal**
   4.1. All trash generated during the procedure should be disposed of in a trash can.
   4.2. Biological waste generate during the procedure should be disposed of in the Biological Waste Container.
   4.3. Laboratories: Only after bleach deactivation should liquid wastes be discharged into the sink or floor drains.
   4.4. Greenhouse: Liquid wastes must be either (a) discharged into the greenhouse floor drains, where it will be collected in the greenhouse effluent tank for further processing, or (b) discharged into the laboratory sinks after bleach deactivation.

5. **Background**
   5.1. Strains received in the Greenhouse from the Main SGI lab will be evaluated and characterized for their ability to grow in a variety of conditions. This protocol represents one of the component tests involved in the assessment of strains for their potential scalability to production scale. The Kill Study provides parameters for cleaning of ponds or photobioreactors at the culmination of a study or experiment.

6. **Materials and Equipment**
   - 1000 µL pipette

- 1000 µL Filtered pipettes
- Pipettor
- 50 mL pipettes
- 50 mL Mini-Bioreactors (x60)
- 500 mL Bottle Top 0.2 µm Filter
- 1 L Polystyrene Storage Bottle
- 500 mL Polystyrene Storage Bottle (x2)
- Proline A
- Proline C
- Urea
- Instant Ocean Sea Mix
- 12 Well Plate (x18)
- Isopropyl Alcohol (70% v/v)
- Muriatic Acid (31% Hydrochloric Acid)
- Chlorine Bleach 10% (sodium hypochlorite)
- Citric acid
- Copper Sulfate
- Hydrogen Peroxide

7. **Procedural Methods**

7.1. Make 1.5 L of PM153 in 35 g/L Instant Ocean Sea Mix

7.2. Filter media through 500 mL Bottle Top 0.2 µm Filter into 1 500 mL Polystyrene Storage Bottle and 1 1 L Polystyrene Storage Bottle

7.3. Make 250 mL 35 g/L Instant Ocean Sea Mix

7.4. Filter Sea Mix through 500 mL Bottle Top 0.2 µm Filter into 1 500 mL Polystyrene Storage Bottle and 1 1 L Polystyrene Storage Bottle

7.5. Prepare Sterile Flow Hood according

7.6. Measure OD_{730} of seed culture to determine amount of stock needed for inoculation according to Formula 1.

$$Amount\ of\ Inoculum = \frac{250}{Seed\ OD730}$$

Formula 1. Inoculum concentration based on Seed Density

7.7. Add *Amount of Inoculum* Determined from *Formula 1* to 250 mL of Sea Mix to dilute inoculum to an OD of 1.0

7.8. Invert bottle to ensure adequate mixing

7.9. Aliquot 3 mL of Diluted Inoculum into 12 wells of a 12 Well Plate

7.10. Add Chlorine Bleach (4.0% sodium hypochlorite)

7.10.1. To 3 wells add 12 µL (4 mL/L)
7.10.2. To 3 wells add 6 µL (2 mL/L)
7.10.3. To 3 wells add 3 µL (1 mL/L)
7.10.4. To 3 wells add No Chlorine bleach (Control)

7.11. Shake plate side-to-side to ensure adequate mixing of inoculum and toxicant

7.12. Incubate plate at room temperature for 4 hours

7.13. Repeat steps 7.9 - 7.11 twice, incubating instead for either 2 or 1 hour each time

7.14. Prepare 12 50 mL Mini-Bioreactors with 22 mL each of PM153 in 35 g/L NaCl

7.15. After incubation, take entire volume of well and inoculate 1 Mini-Bioreactor each with a single well of inoculum

7.16. Incubate Mini-Bioreactors in Greenhouse Conviron box maintained with the following conditions:

- 25° C
- 1 ppm CO_2
- 12-hour light/dark cycles
- Shaking at 120 rpm

7.17. Monitor for growth after 1 week